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bacterial cell wall, thus enabling progeny phage release. When exogenously added, these enzymes lyse the peptidoglycan of Gram-positive pathogens, resulting in osmotic lysis and cell death. One particular endolysin, PlyC, has a distinctive ability to translocate eukaryotic membranes and retain killing activity in the intracellular environment, making it a particularly interesting target molecule for the development of novel therapeutic approaches. However, the protein-membrane interactions and the cell penetration processes remain unknown. Here, we investigate molecular-scale aspects of such membrane interactions using sparsely-tethered lipid bilayer membranes (stBLMs), a robust planar biomimetic lipid membrane model.

Applying complementary surface-sensitive techniques such as surface plasmon resonance, electrochemical impedance and neutron reflectometry, we demonstrate the first steps towards a mechanistic understanding of how the PlyC binding domain, PlyCB, initiates membrane translocation. Our data reveals that while the interaction of PlyCB with purely zwitterionic membranes is negligible, the protein strongly interacts with anionic membranes that contain phosphatidylserine (PS) above a well-defined concentration threshold. In contrast, PlyCB affinity for other anionic lipids tested is low, suggesting specificity for PS rather than non-specific ionic interactions. Furthermore, the PlyCB point mutant R66E that lacks the ability to translocate membranes has likewise lost affinity for PS. With Neutron reflection we identified two distinct PlyCB membrane-association modes. Depending on PS membrane concentration, PlyCB is either peripherally associated or membrane-spanning. Because the outer leaflet of eukaryotic membranes is largely zwitterionic, our findings imply that PlyC induces and/or recognizes PS exposure during cellular uptake. In addition, these results show how lipid membrane surface charge density and composition play a critical role for PlyC internalization.

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Structural Basis of Phosphoinositide (PIP) Recognition by the TIRAP PIP-Binding Motif

Xiaolin Zhao[1], Shuyan Xiao[1], Sam Berk[1], Anne M. Brown[2], David R. Bevan[2], Geoffrey Armstrong[3], Daniel G.S. Capelluto[1].
[1]Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA, [2]Department of Biochemistry, Virginia Tech, Blacksburg, VA, USA, [3]Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO, USA.

Toll-like receptors (TLRs) provide early immune system recognition and response to infection. TLRs activated by pathogens consequentially initiate a cytoplasmic signaling cascade though adaptor proteins, the first being the modular TIR domain-containing adaptor protein (TIRAP). TIRAP contains a C-terminal TIR domain, which is responsible for association with TLRs and other adaptors including the myeloid differentiation primary response gene88 (MyD88) protein. Membrane recruitment of TIRAP is mediated by its N-terminal PIP-binding motif (PBM). Upon ligand-mediated activation, TLRs are recruited to the PIP-enriched regions where TIRAP resides. At these sites, TIRAP recruits MyD88 to the membrane by bridging MyD88 to activate the TLR signaling pathway. To understand the mechanism of membrane targeting of TIRAP and the basis for its regulation, we functionally and structurally characterized its PBM using experimental and computational studies. TIRAP PBM adopts a folded conformation in membrane mimics, such as dodecylphosphocholine micelles, and binds PIPs. Structural rearrangements of TIRAP PBM were influenced by membrane interaction, with monodispersed PIPs inducing helical structure in the peptide. In contrast, monodispersed phosphatidylinositol and inositol trisphosphate did not promote structural changes in TIRAP PBM. NMR spectra reveal that TIRAP PBM binds PIPs in a fast exchange regime with a moderate affinity through two conserved basic regions. Solution NMR structure of TIRAP PBM shows a central short helix, and paramagnetic studies indicate that this region is close to the micelle core. Molecular dynamics simulations studies indicated that TIRAP PBM diffused to and interacted with a model membrane composed of palmitoyl oleoyl phosphatidylcholine and phosphatidylinositol 4,5-bisphosphate. Thus, we propose that two sets of basic residues contact both the head group and acyl chains of PIPs, whereas the central helix is responsible for membrane insertion.

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Mechanism of Action of Salt Adaptation Mutations in Artemia Franciscana

Jessica Eastman, Sukanyalakshmi Chebrolu, Pablo Artigas.
Texas Tech Health Sciences Center, Lubbock, TX, USA.

Nearly all animals maintain a large electrochemical gradient for Na^+ across the plasma membrane. This gradient is generated by the Na-K pump, which exports 3 Na^+ and imports 2 K^+ per ATP molecule hydrolyzed. Ion-coordinating residues in the α subunit are usually conserved, but the brine shrimp (Artemia franciscana) living in extreme saline conditions express a pump with two asparagine to lysine substitutions within the ion binding site region (Jorgensen and Amat (2008) J. Memb Biol. 221:39-49). We used two-electrode voltage clamp on Na^+-loaded Xenopus oocytes to evaluate the effect of the substitutions (N333K and N785K) individually and concurrently on the function of Xenopus Na/K pumps. We studied their effect on activation of pump currents by eternal K^+ and on voltage-dependent conformational changes related to external Na^+ binding (charge movement). The center of the Q-V curves are displaced by ~-80 mV by both individual mutations suggesting a reduced (>10 fold) external Na^+ affinity. Surprisingly the double mutant showed a nearly identical shift in the Q-V, indicating non-additive effects on external Na^+ affinity. Apparent affinity for K^+ in the absence of Na^+ was reduced (~10-fold) by the N785K mutation while N333K and the double mutant had similar affinity to the wild type. These results can be explained with recent structures of the Na/K pump with Na^+ or K^+ bound. N333, outside the ion-binding pocket, forms a hydrogen bond with ion-coordinating N785 in the Na^+ bound conformation. Once the disruption of normal Na^+ coordination by N785K is in place the mutation N333K does not affect Na^+ binding. This contrasts with previous findings regarding internal Na^+ binding. We are investigating intracellular ion dependence in these mutants by measuring currents in patch clamp and enzymatic activity in membrane preparations. Supported by NSF-MCB-1243842 & NIH-NS081570-01.

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Determining Oligomeric Order of a Membrane Protein by Double Electron-Electron Resonance Spectroscopy

Sergey Milikisiyants[1], Shenlin Wang[2], Rachel Munro[3], Matthew Donohue[1], Leonid S. Brown[3], Tatyana I. Smirnova[1], Vladimir Ladizhansky[3], **Alex I. Smirnov**[1].
[1]Chemistry, North Carolina State University, Raleigh, NC, USA, [2]Beijing Nuclear Magnetic Resonance Center and College of Chemistry and Molecular Engineering, Peking University, Beijing, China, [3]Physics and Biophysics Interdepartmental Group, University of Guelph, Guelph, ON, Canada.

Many different classes of membrane proteins are known to form oligomers in cellular membranes in order to carry out specific cellular functions. Detection and detailed structural characterization of protein oligomers in lipid milieu is by no means a trivial task. Here we demonstrate the use of spin-labeling and Double Electron-Electron Resonance (DEER) spectroscopy to determine the oligomeric order of a membrane protein. Specifically, we investigate oligomerization of a seven-helical membrane photoreceptor Anabaena Sensory Rhodopsin (ASR) from Anabaena sp. PCC7120. Recently, ASR structure has been solved by both x-ray protein crystallography (Science 2004, 306, 1390) and solid-state NMR (Nat Methods 2013, 10, 1007). Here we show that the same spin-labeling sites we employed for paramagnetic relaxation enhancement (PRE) NMR can also be used for DEER experiments. The results demonstrate that DEER restraints can not only differentiate between the dimer (x-ray) and trimer (ssNMR) models that have very different interfaces, but further rule out hypothetical tetramer and other higher order polygon models. The crux of our DEER-based approach relies on taking advantage of the multi-spin effects and analyzing experimental DEER traces by direct fitting to the multispin models. Overall, the observed profound effect of higher order spin correlations on the DEER trace allows for a reliable differentiation between oligomer models. In the specific case of ASR, the DEER trace modeling allowed us to unambiguously discard all but the trimer model. Furthermore, the addition of DEER electron-electron distances to the NMR restraints in the structure calculation protocol improves local RMSD, and allows for refinement of the orientation of helices. Supported by U.S. DOE Contract DE-FG02-02ER15354 to AIS and NSERC Discovery Grants RGPIN-2014-04547 to VL and RGPIN-2013-250202 to LSB.

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Nucleotide-Dependent Membrane Interaction and Dimerization of K-Ras4B

Hyunbum Jang[1,2], Shaoyong Lu[2,3], Mayukh Chakrabarti[2,4], Lyuba Khavrutskii[1,2], Nadya I. Tarasova[2], Vadim Gaponenko[5], Ruth Nussinov[1,2].
[1]Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Lab, Frederick, MD, USA, [2]Cancer and Inflammation Program, National Cancer Institute at Frederick, Frederick, MD, USA, [3]Shanghai JiaoTong University, Shanghai, China, [4]Department of Biotechnology, Johns Hopkins University, Baltimore, MD, USA, [5]Departments of Medicinal Chemistry and Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL, USA.